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ORIGINAL ARTICLE

# Optimization of biosurfactant production by *Streptomyces* isolated from Egyptian arid soil using Plackett–Burman design

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**KEYWORDS**

*Streptomyces*;
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Arid soil;
Plackett–Burman design

**Abstract** Surfactants are amphipathic molecules which reduce surface tension, widely used in pharmaceutical, cosmetic and food industries. The current study aimed to isolate *Streptomyces* species from Egyptian arid soil, efficient in biosurfactant production. Fifty isolates of *Streptomyces* were obtained from arid soil in Egypt. Primary screening, using lipase, drop collapse, parafilm M and haemolysis tests, showed 37 isolates (74%) having biosurfactant activity. Secondary screening resulted in 17 isolates based on their activities using emulsification index method. Isolate 5S was selected as the most efficient in biosurfactant production, having emulsification index equal to 31.74%. Plackett–Burman design was adopted to evaluate the factors affecting biosurfactant production. Among the tested nine variables of starch nitrate medium, treated molasses, peptone, Tween 80, incubation period and inoculum size were identified as significant factors affecting biosurfactant production. Cultivation of isolate 5S in the optimized medium elevated biosurfactant production to 42.68% (according to emulsification index) from 31.74%, obtained in un-optimized medium.



## Introduction

Oil pollution and remediation technology has become of global increasing interest. One of the major sources of environment pollution is hydrocarbons. Most of the hydrocarbons are insoluble in water and their degradation using microorganisms has an important role in combating environmental pollution. One of the mechanisms used by these microorganisms for hydrocarbon degradation is through production of extracellular biosurfactant (Kalyani et al., 2014).

Compared to their chemically synthesized counterpart, biosurfactants possess many advantages including their diversity, environment-friendly nature, suitability for large-scale production and selectivity (De Quadros et al., 2011). Moreover, and unlike synthetic surfactants, biosurfactants are easily biodegradable and thus particularly suited and more preferable for environmental applications such as bioremediation and dispersion of oil spills.

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**Table 1** Site descriptions of soils samples.

| Samples no. | Location | Colonies obtained | Governorate | Latitudes (°) | Longitudes (°) |
|---|---|---|---|---|---|
| 1 | Bahariya Oases | 3 | Giza | 29.084 | 28.390 |
| 2 | Mines | 2 | Giza | 28.943 | 28.281 |
| 3 | Bawiti | 2 | Giza | 28.766 | 28.333 |
| 4 | Peace | 3 | Giza | 28.800 | 28.301 |
| 5 | Black Desert | 4 | New valley | 28.386 | 27.608 |
| 6 | White Desert | 2 | New valley | 28.454 | 27.677 |
| 7 | Farafra 1 | 3 | New valley | 27.984 | 27.219 |
| 8 | Major General Sabih | 3 | New valley | 27.734 | 26.491 |
| 9 | Abo-horairah | 2 | New valley | 27.650 | 26.499 |
| 10 | Abu monkar | 2 | New valley | 27.598 | 26.495 |
| 11 | Mountains Negev 1 | 2 | New valley | 27.601 | 26.494 |
| 12 | Great Sand Sea | 5 | New valley | 27.665 | 26.537 |
| 13 | Mountains Negev 2 | 4 | New valley | 27.667 | 26.493 |
| 14 | Paris 1 | 2 | New valley | 31.281 | 28.113 |
| 15 | Kharga Oases | 3 | New valley | 31.082 | 28.211 |
| 16 | Sohag (army road) | 2 | Sohag | 31.324 | 27.330 |
| 17 | Assiut (army road) | 2 | Assiut | 31.014 | 27.015 |
| 18 | Minya | 2 | Minya | 30.448 | 25.314 |
| 19 | Bani Suef | 2 | Bani Suef | 30.466 | 25.513 |
| Total | | 50 | | | |

Research in the area of biosurfactants has expanded in recent years due to its potential use in different areas, such as food industry, agriculture, pharmaceuticals, oil industry, petrochemicals, and paper and pulp industry. The development of this research line has also great importance for environmental protection (Karsa et al., 1999; Meylheuc et al., 2001).

In this context, the search for promising biosurfactant producing microorganisms requires untraditional source such as arid soil. Arid soil is the soil which has almost no water available for soil formation and the growth of mesophytic plants for long period. Such soil covers approximately one-third of the earth surface. Arid and semi-arid soils are mainly found

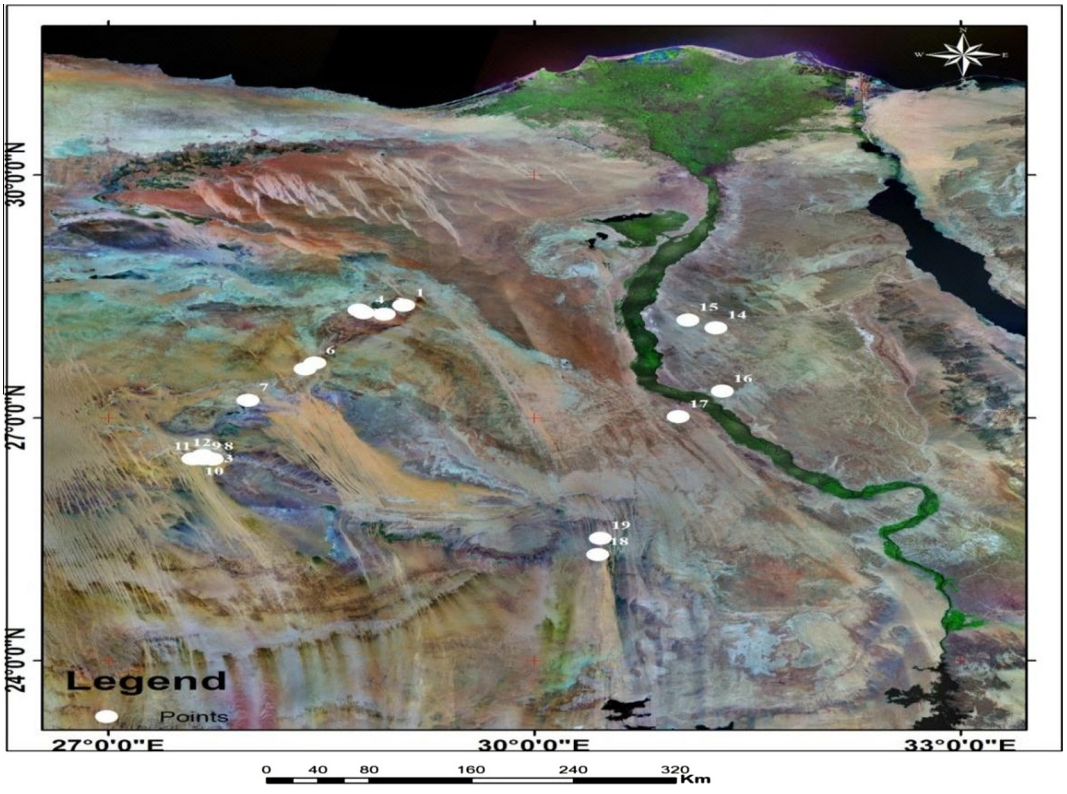


**Fig. 1** Distribution of samples locations. *Source:* National Authority for Remote Sensing and Space Sciences (NARSS), Cairo, Egypt.

**Table 2** Variables affecting biosurfactant production by *Streptomyces* sp. for the Plackett–Burman experiment.

| Nutritional and environmental factors | Level of variables in design | |
|---|---|---|
| | −1 | +1 |
| Treated molasses (g/l) | 15 | 25 |
| Peptone (g/l) | 1 | 3 |
| Waste oil (%) | 1 | 3 |
| Petrol (%) | 1 | 3 |
| Tween 80 (%) | 1 | 3 |
| Temperature (°C) | 25 | 35 |
| Agitation (rpm) | 50 | 150 |
| Inoculum size (%) | 1 | 3 |
| Incubation periods (day) | 3 | 7 |
| pH | 6 | 8 |

in Africa, The Middle East, North and South America and Australia (Verheye, 2012).

Microorganisms inhabiting arid soil, especially actinomycetes are essential for the proper functioning and sustainability of its ecosystems (Choudhary et al., 2011). Most actinomycete species have the capability to synthesize many different biologically active secondary metabolites, such as antibiotics, herbicides, pesticides, biosurfactant, anti-parasitic substances and enzyme inhibitors (Tanaka and Omura, 1990). The genus *Streptomyces* was found to produce many active biosurfactants (Manivasagan et al., 2014).

Therefore, the current study focused on development of an optimized medium for biosurfactant production by a selected arid-soil-adapted *Streptomyces* isolate. Optimization was carried out using Plackett–Burman experimental design to maximize the biosurfactant production by a selected *Streptomyces* isolate.

## Materials and methods

### *Soil samples*

Nineteen soil samples were collected from 19 different arid locations in Egyptian desert (Table 1 and Fig. 1). For isolation of *Streptomyces* species, 10 g of each sample was suspended in 90 ml sterile distilled water and serially diluted under sterile conditions. One ml of each suspension was spread on the surface of sterile starch nitrate agar medium plates (Waksman and Lechevalier, 1961) and incubated at 30 ± 2 °C for 7, 14 and 21 days. Resulted colonies were used for the primary screening.

### *Isolation and purification of streptomycetes*

Grown actinomycetes colonies were picked up by sterilized inoculating needle and maintained on the same medium at 4 °C for further studies (Waksman and Lechevalier, 1961). Purified isolates were identified up to genus according to their cultural and morphological characteristics (Pridham and Tresner, 1974).

### *Inoculum preparation*

Spore suspension of the selected streptomycetes isolate was prepared by growing the selected isolate on starch nitrate agar medium plate (90 mm diameter) for 15 days at 30 ± 2 °C and then spores were collected by scrapping the spores using alcohol-sterilized blade. Spores were resuspended in 50 ml of sterile saline water (0.9% NaCl) by scratching the whole surface of agar plates. Spores concentration ($25 \times 10^8$ spores/ml) was determined by haemocytometer slide (Trejo-Estrada et al., 1998).

### *Primary and secondary screening for biosurfactant activity*

Biosurfactant activity of the pure culture of streptomycetes was determined by five different methods: (1) Haemolysis, (2) Drop collapsing test, (3) Lipase production, (4) Para film M test, and (5) Emulsification measurement. Primary screening included haemolysis, drop collapsing test, lipase production, and Para film M test, while the secondary screening included emulsification measurement.

### *Haemolytic activity*

Haemolysis was carried out on plates of blood agar medium supplemented with human blood (5%). The blood agar plates

**Table 3** Plackett–Burman experimental design for ten variables with different levels for biosurfactant production by *Streptomyces* isolate.

| Run | Nutritional factors | | | | | Environmental factors | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Molasses treated (g/l) | Peptone (g/l) | Waste oil (%) | Petrol (%) | Tween 80 (%) | Temp. (°C) | Agitation (rpm) | Inoculum size (%) | Incub. period (day) | pH |
| 1 | 25 | 3 | 1 | 3 | 3 | 35 | 50 | 1 | 3 | 8 |
| 2 | 25 | 3 | 1 | 1 | 1 | 35 | 50 | 3 | 7 | 6 |
| 3 | 15 | 1 | 1 | 3 | 3 | 35 | 150 | 1 | 7 | 6 |
| 4 | 25 | 3 | 3 | 1 | 3 | 25 | 150 | 1 | 7 | 6 |
| 5 | 25 | 1 | 1 | 1 | 3 | 25 | 150 | 3 | 3 | 8 |
| 6 | 15 | 3 | 3 | 1 | 1 | 35 | 150 | 1 | 3 | 8 |
| 7 | 25 | 1 | 3 | 3 | 1 | 35 | 150 | 3 | 3 | 6 |
| 8 | 15 | 3 | 1 | 3 | 1 | 25 | 150 | 3 | 7 | 8 |
| 9 | 15 | 1 | 1 | 1 | 1 | 25 | 50 | 1 | 3 | 6 |
| 10 | 25 | 1 | 3 | 3 | 1 | 25 | 50 | 1 | 7 | 8 |
| 11 | 15 | 1 | 3 | 1 | 3 | 35 | 50 | 3 | 7 | 8 |
| 12 | 15 | 3 | 3 | 3 | 3 | 25 | 50 | 3 | 3 | 6 |

**Table 4** Primary screening for biosurfactant production by *Streptomyces* isolates.

| Isolates no. | Colour of aerial mycelium | Drop collapsing test* | Para film M test** | Haemolysis (mm) | Lipase production (mm) |
|---|---|---|---|---|---|
| 6s | Grey | + | + | 0.2 | 1.5 |
| M70 | Grey | + | + | 0.1 | 0.2 |
| 5S | Grey | + | + | 0.6 | 1.8 |
| 9G$^-$ | Grey | + | + | 0.1 | 1.3 |
| F18 | Grey | + | + | 0.1 | 0.3 |
| 50 | Grey | – | – | 0.4 | 0 |
| 13 | Grey | + | + | 0.1 | 0.3 |
| 4SS | Grey | + | + | 0.2 | 0.2 |
| Q4 | Grey | + | + | 0.5 | 1.5 |
| Q2 | Grey | + | + | 0.4 | 1.3 |
| Q6 | Grey | + | + | 0.1 | 0.4 |
| Q1 | Grey | + | + | 0.3 | 1.7 |
| Q3 | Grey | + | + | 0.1 | 0.3 |
| 5S1 | Grey | + | + | 0.1 | 0.6 |
| BLUE | Grey | – | – | 0.1 | 0 |
| 12 | Grey | + | + | 0.1 | 0.2 |
| 9 | Grey | + | + | 0.5 | 1.2 |
| F86 | Grey | + | + | 0.2 | 0.7 |
| GRAY | Grey | – | – | 0.1 | 0 |
| M70ST | Grey | – | – | 0.1 | 0 |
| 9G | Grey | + | + | 0.2 | 0.4 |
| 6S$^-$ | Grey | + | + | 0.4 | 06 |
| L13 ST | Grey | + | + | 0.6 | 1.2 |
| 19 | Grey | + | + | 0.2 | 0.8 |
| 64 ST | Grey | + | + | 0.1 | 0.5 |
| 12 ST | Grey | + | + | 0.5 | 1.3 |
| 15$^-$ | Grey | + | + | 0.4 | 2 |
| READ | Grey | – | – | 0.1 | 0 |
| L13 | Grey | + | + | 0.9 | 1.3 |
| F7 | Grey | – | – | 0.1 | 0 |
| F16 | Grey | – | – | 0.2 | 0 |
| F12 | White | + | + | 0.1 | 0.4 |
| F1 | White | + | + | 0.1 | 0.5 |
| LOB ST | White | + | + | 0.2 | 0.9 |
| 4S | White | + | + | 0.1 | 1.2 |
| L | White | + | + | 0.4 | 0.9 |
| 14S$^-$ | White | + | + | 0.2 | 1.5 |

Total isolates: 37, (+) *means drop was flat, (+) **means spreading of drop. The remaining 13 isolates showed no biosurfactant productions.

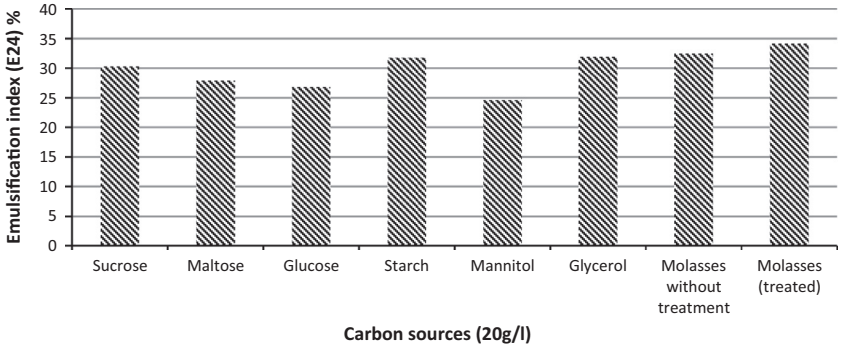


**Fig. 2** Effect of carbon sources on biosurfactant production by *Streptomyces* isolate 5S.

were inoculated and incubated at 30 °C for 7 days. Plates were examined for clear zone around the colonies (Carillo et al., 1996).

*Drop collapsing test*

Biosurfactant production was determined using the qualitative drop-collapse test described by Youssef et al. (2004). Mineral

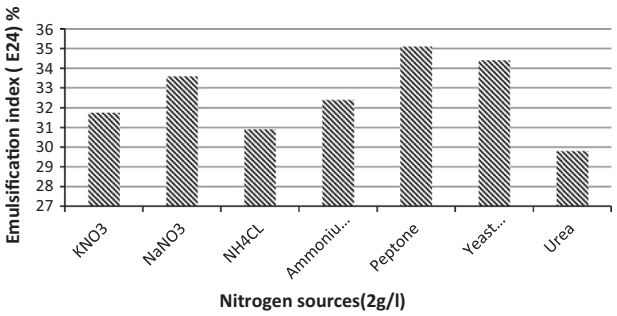


**Fig. 3** Effect of nitrogen sources on biosurfactant production by *Streptomyces* isolate 5S.

oil (2 µl) was added to 96-well microtiter plates, 5 µl of the streptomycete cultures supernatant was added to the surface of the oil in the well and plates were incubated for 1 h at 37 °C. The shape of drop on the oil surface was observed after 1 min. The culture supernatant makes the drop collapsed, it indicates as a positive result and if the drops remain intact, it indicates as a negative result. Distilled water was used as control treatment.

*Lipase production*

Lipase produced by the pure streptomycetes cultures was determined using tributyrin agar plates. Plates were inoculated and incubated at 30 °C for 7 days. The plates were examined for clear zone around the colonies after 7 days (Gandhimathi et al., 2009).

*Para film M test*

Ten µl of the selected isolate culture-supernatants was mixed with 1% bromothymol blue, added to the hydrophobic surface of the parafilm M. The shape of this drop on the surface was inspected after 1 min. The diameters of these droplets are evaluated. Sodium lauryl sulphate and distilled water were used as positive and negative controls, respectively. If the drop becomes flat, it indicates the presence of biosurfactant. If it remains in a dome shape, it indicates the absence of biosurfactant (Youssef et al., 2004).

**Table 5** Results for the secondary screening for biosurfactant producing *Streptomyces* isolates.

| Isolates no. | Sodium chloride tolerance up to (%) | Emulsification index (E24) (%) |
|---|---|---|
| 12 ST | 3 | 27.9 |
| 14S$^{-}$ | 2 | 22.5 |
| Q1 | 4 | 20.8 |
| Q2 | 3 | 29.4 |
| Q4 | 1 | 22.5 |
| 4S | 5 | 23.3 |
| 9G$^{-}$ | 6 | 23.1 |
| 6s | 4 | 30.8 |
| **5S** | **6** | **31.74** |
| 9 | 4 | 30.3 |
| L13 ST | 1 | 27.5 |
| 12 ST | 1 | 25.2 |
| L13 | 0 | 23.5 |
| 50 | 3 | 20.3 |
| Q9 | 4 | 15.2 |
| L | 6 | 26.6 |
| 6S$^{-}$ | 3 | 14.7 |
| 15$^{-}$ | 5 | 17.1 |

Bold values indicate isolate giving the highest record in emulsification index.

*Emulsification measurement*

The emulsifying capacity was evaluated by an emulsification index (E24). The E24 of culture samples was determined by adding 2 ml of paraffin oil and 2 ml of the cell free broth in test tube, vortexed at high speed for 2 min and allowed to stand for 24 h. The percentage of emulsification index was calculated using the following equation (Abouseoud et al., 2008):

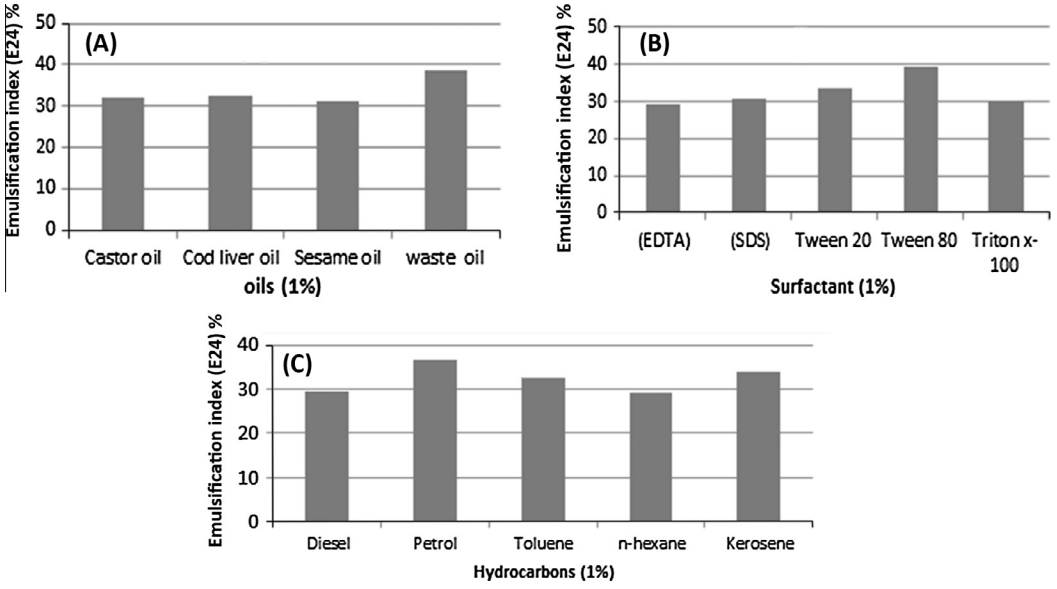


**Fig. 4** Effect of different oils (A), surfactants (B) and hydrocarbons (C) on biosurfactant production by *Streptomyces* isolates 5S.

**Table 6** Actual and predictive emulsification index (E24) for *Streptomyces* isolate 5S as affected by different nutritional and environmental factors.

| Run | Treated molasses (g/l) | Peptone (g/l) | Waste oil (%) | Petrol (%) | pH | Tween 80 (%) | Temp. (°C) | Agitation (rpm) | Inoculum size (%) | Incub. periods (day) | Emulsification index (E24) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Actual | Predictive |
| 1 | 25 | 3 | 1 | 3 | 8 | 3 | 35 | 50 | 1 | 3 | 36.19 | 36.25 |
| 2 | 25 | 3 | 1 | 1 | 6 | 1 | 35 | 50 | 3 | 7 | 33.74 | 33.76 |
| 3 | 15 | 1 | 1 | 3 | 6 | 3 | 35 | 150 | 1 | 7 | 29.74 | 29.76 |
| 4 | 25 | 3 | 3 | 1 | 6 | 3 | 25 | 150 | 1 | 7 | 32.52 | 32.46 |
| 5 | 25 | 1 | 1 | 1 | 8 | 3 | 25 | 150 | 3 | 3 | 39.98 | 39.82 |
| 6 | 15 | 3 | 3 | 1 | 7 | 1 | 35 | 150 | 1 | 3 | 31.06 | 31.26 |
| 7 | 25 | 1 | 3 | 3 | 6 | 1 | 35 | 150 | 3 | 3 | 42.52 | 42.68 |
| 8 | 15 | 3 | 1 | 3 | 8 | 1 | 25 | 150 | 3 | 7 | 32.11 | 31.95 |
| 9 | 15 | 1 | 1 | 1 | 6 | 1 | 25 | 50 | 1 | 3 | 35.23 | 35.03 |
| 10 | 25 | 1 | 3 | 3 | 8 | 1 | 25 | 50 | 1 | 7 | 37.33 | 37.31 |
| 11 | 15 | 1 | 3 | 1 | 8 | 3 | 35 | 50 | 3 | 7 | 32.19 | 32.39 |
| 12 | 15 | 3 | 3 | 3 | 6 | 3 | 25 | 50 | 3 | 3 | 30.06 | 30.00 |

**Table 7** Linear regression coefficients, corresponding *F*-values and *p*-values for biosurfactant production by *Streptomyces* isolate 5S by the Plackett–Burman design experiment.

| Variable | Co-efficient | *F*-value | *p*-Value |
|---|---|---|---|
| Model | 34.43 | 190.12 | 0.0052 |
| (A) Molasses | 2.62 | 796.88 | 0.0013 |
| (B) Peptone | −1.74 | 350.49 | 0.0028 |
| (D) Petrol | 0.23 | 6.12 | 0.1318 |
| (E) pH | 0.48 | 22.77 | 0.0412 |
| (G) Agitation | 0.31 | 10.86 | 0.0811 |
| (H) Inoculum size | 0.67 | 52.37 | 0.0186 |
| (J) Incubation period | −1.49 | 258.37 | 0.0038 |
| (K) Tween 80 | −0.98 | 112.19 | 0.0088 |
| (L) Dummy | −0.70 | 56.62 | 0.0172 |

Values of "Prob > *F*" less than 0.0500 indicate model terms are significant.

$$\text{E24} = \text{Height of emulsion formed (cm)} \times 100/\text{Total height of solution (cm)}.$$

*Optimization of biosurfactant production*

*Streptomyces* isolate with the highest biosurfactant production was grown on the starch nitrate medium (Waksman and Lechevalier, 1961), and utilized in the following set of experiments.

*Effect of different carbon sources*

Starch as a carbon source was replaced with glucose, sucrose, molasses (untreated), molasses (treated), maltose, glycerol and mannitol at concentrate of 20 g/l for each. Carbon sources were separately sterilized by filtration (through bacteriological filters) then added to the production medium under aseptic conditions. Fermentations were carried out in 250 ml Erlenmeyer flasks with 50 ml of medium, inoculated with 1% of spore suspension (previously described) and incubated at 30 °C for 3 days at agitation speed of 100 rpm.

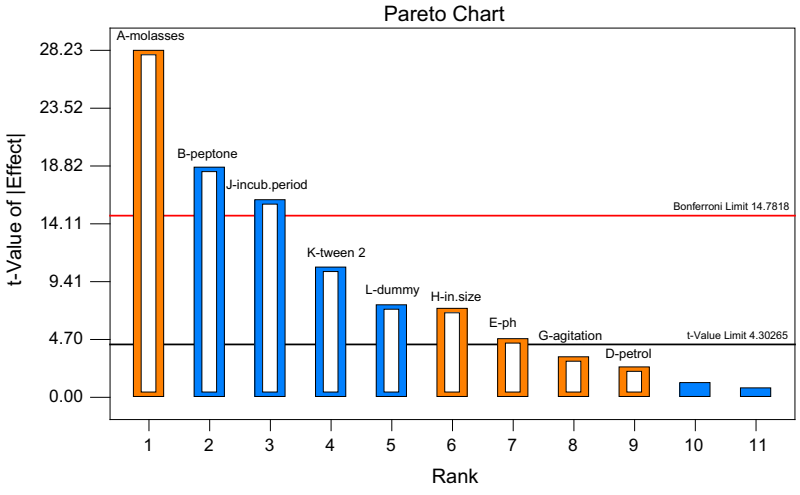


**Fig. 5** Pareto chart showing the effect of media components on biosurfactant production.

Molasses treatment was conducted as follows: sugarcane molasses (85% solid content) were diluted two-fold (w/v) with distilled water, pH was adjusted to 2.0 with 1 N HCl, then the solution was held in boiling water bath for 40 min for sucrose hydrolysis. After hydrolysis, the solution was cooled to room temperature and the pH was adjusted to 6.0 with 1 N NaOH. The precipitate was removed by centrifugation and the supernatant was used in the production medium as described above (Bhosale and Gadre, 2001).

*Effect of different nitrogen sources*

Nitrogen sources such as $(NH_4)_2SO_4$, $KNO_3$, $NaNO_3$, $NH_4Cl$, Peptone, Yeast extract and Urea were separately added to starch nitrate medium at a concentration of 2 g/l instead of $KNO_3$. Fermentations were carried out in 250 ml Erlenmeyer flasks with 50 ml of medium and incubated at 30 °C for 3 days at agitation speed of 100 rpm.

*Effect of oils, surfactants and hydrocarbons on biosurfactant production*

To study the effect of crude oil and surfactants on biosurfactant production, different oils (castor oil, cod-liver oil, sesame oil and waste oil) were added separately to starch nitrate medium at concentration of 2% (v/v). Surfactants (EDTA, SDS, Tween 20 and Tween 80) were added separately to starch nitrate medium at concentration of 1% (v/v). The emulsification activity of medium was measured. The effect of different hydrocarbons (diesel, petrol, toluene, xylene and kerosene) at concentration of 1% on biosurfactant production was also studied (Kokare et al., 2007 and Chakraborty et al., 2009).

*Optimization of growth medium using Plackett–Burman experimental design*

Ten factors, namely treated molasses, peptone, waste oil, petrol, Tween 80, pH, temperature, agitation rate, inoculum size and incubation periods were selected for this experiment. The selection of nutrient for Plackett–Burman experimental design was performed by the fermentation of medium optimization (Salam et al., 2013). The Plackett–Burman experimental design for the above ten variables (Table 2) was used to evaluate the relative importance of various nutrients for biosurfactant production in shake flask culture and experimental design was prepared with the help of software Design Expert trial 9.0.4.1 (Stat Ease Inc., USA). In Table 3, each row represents an experiment (run) and each column represents a different variable. For each nutrient variable, two different concentrations high (+) and low (−) were tested (Table 2). All experiments have been carried out in triplicate in 250 ml Erlenmeyer flasks containing 50 ml medium.

*Statistical analysis of the Plackett–Burman design*

Experimental data were analysed by the standard methods of Plackett–Burman and software Design Expert trial 9.0.4.1 (Stat Ease Inc., USA). The effect of each variable was determined. The percentage of contribution of each nutrient parameter was calculated. Mean squares of each variable (the variance of effect) were calculated. The experimental error was calculated by averaging the mean squares of the dummy variables. Factors showing larger effects were identified using *F*-test.

*Statistical analysis of the simple experiment*

All data obtained were exposed to the proper statistical analysis according to Snedecor and Cochran (1991) using Costat computer program V 6.303 (2004). LSR at 5% level was used to differentiate between means.

## Results and discussion

*Isolation and purification of actinomycetes*

Fifty actinomycete isolates were obtained from arid soils of 19 different localities in Egyptian desert (Table 1), purified and maintained on the starch nitrate agar medium for further studies. According to their cultural and morphological characteristics, the obtained actinomycete isolates were identified as members of genus *Streptomyces* as they form well developed branching, non-septate, non-fragmented aerial mycelia bearing the long non-motile spore chains, not borne in verticillate sporophores (Pridham and Tresner, 1974).

*Primary and secondary screening of biosurfactant production of Streptomyces isolates*

A total of fifty *Streptomyces* isolates was screened for biosurfactant production by cultivation on starch nitrate broth medium then conducting a drop collapsing test. A positive result is identified by a flat drop around the colonies of the isolates, indicating a biosurfactant activity. Inoculation of the isolates on blood agar plate produced a clear zone around the colonies, indicating the biosurfactant activity by the surface active molecules produced by *Streptomyces* sp. Inoculation of the positive-result isolates on Tributyrin agar plate produced a clear zone, which indicates production of lipase enzyme. Positive results for parafilm-M test were indicated by a flat drop in the hydro-phobic surface of the parafilm M. Out of the 50 *Streptomyces* isolates, 37 showed biosurfactant activities, representing 74% of all isolates (Table 4).

Out of 37 *Streptomyces* isolates, 18 isolates were selected for secondary screening using emulsification index test on the basis of the highest zone diameter on lipase and haemolysis test. Results in Table 5 indicate that *Streptomyces* isolate 5S showed high biosurfactant activity, giving emulsification index 31.74% and can tolerate NaCl up to 6%, so it was selected for further studies.

*Optimization of growth medium*

As the most efficient in biosurfactant production, isolate 5S was used for the optimization of the biosurfactant production. Growth medium was optimized by testing various carbon and nitrogen sources, different oils, surfactants and hydrocarbon compounds.

*Effect of carbon and nitrogen sources*

The production of biosurfactant was found to be dependent on the composition of the medium. Results given in Fig. 2 indicate that *Streptomyces* sp. 5S was able to utilize different carbon sources used for growth and production of biosurfactant. In shake-flask experiments, it was found that biosurfactant secretion was dependent of carbon source. Eight different carbon

sources screened for their effects on the biosurfactant production, out of these eight sources molasses in either treated or untreated form were found favourable. Data in Fig. 2 also reveal that the highest significant biosurfactant production was achieved using treated molasses having E24 34.1%. Similar studies of Khopade et al. (2012) found that the maximum biosurfactant production by *Streptomyces* sp. was obtained by using sucrose as a sole carbon source.

The effect of nitrogen source on the biosurfactant production is shown in Fig. 3. Data showed that significant biosurfactant production by *Streptomyces* isolate 5S favoured peptone as a nitrogen source, having E24 of 35.11%. Similar results were obtained by other studies where organic sources of nitrogen were preferred over inorganic ones (Abouseoud et al., 2008; Kalyani et al., 2014).

### *Effect of oils, surfactants and hydrocarbons on production of biosurfactant by Streptomyces sp. 5S*

Fermentation was carried out with addition of different types of oils, surfactants and hydrocarbons in the fermentation medium. Data given in Fig. 4(A, B and C) clearly show that *Streptomyces* isolate 5S was able to hydrolyse different oils, surfactants and hydrocarbon compounds. Out of hydrocarbons, petrol gave maximum biosurfactant production of 36.78% after 3 days of incubation, while waste oil was the best in oils and Tween 80 was the best in surfactants, giving production of 38.83% and 39.1%, respectively. Kokare et al. (2007) found that, out of all tested oils and hydrocarbons, toluene (1% v/v) induced maximum biosurfactant activity by *Streptomyces* sp.

From the abovementioned results, it can be concluded that treated molasses (2%) and peptone (0.2%) were the best carbon and nitrogen compounds added to starch nitrate medium compared to starch (2%) and $KNO_3$ (0.2%) for the production of biosurfactant. This basal medium will be used for subsequent studies.

### *Optimization of biosurfactant production by Streptomyces sp. 5S using Plackett–Burman design*

Plackett–Burman design is one of the screening designs used for identifying significant factors among many potential factors. In this approach, usually only main effects are estimated. In the traditional method, screening for each category of the sources is done at an arbitrarily selected level of each source, one category at a time, while keeping the other category constant, again at arbitrarily selected levels. In Plackett–Burman design, generated data are used to select few compounds in each category, based on highest product promotion. Different levels of the selected category are then evaluated to achieve optimum level. The interactive effects among the sources of different categories are completely ignored. So, statistical experimental designs are powerful tools for searching the key factors rapidly from a multivariable system and minimizing the error in determining the effect of the categories. Therefore, results are achieved in an economical manner (Kalyani et al., 2014).

In this part of study, Plackett–Burman design was employed to evaluate the effect of ten different culture elements on the production of biosurfactant by *Streptomyces* sp. 5S using a basal medium, indicated that there was a variation of biosurfactant production in the range from 29.74% to 42.52% (Table 6). The main effects (*t*-values and *p*-values) were estimated for each independent variable on biosurfactant production as shown in Table 7. The results indicated that the presence of high levels of peptone, treated molasses, Tween 80, pH, incubation periods and inoculum size in the basal medium positively affected biosurfactant production. These results are confirmed from the **Pareto chart** as shown in Fig. 5, which indicates higher effects presented in the upper portion and then progress down to the lower effects. The correlation coefficient ($R^2$) was found to be 0.9936, showing good fitness of the model, and given that "Predictive R-Squared" was 0.9560, which concluded that these values are in reasonable agreement with the "Adjustive R-Squared" of 0.9936 (with difference less than 0.2), indicating that a good agreement between the experimental and predicted values on biosurfactant production.

Results of ANOVA test and calculated *t*-test showed that treated molasses, incubation periods, Tween 80, inoculum size and peptone were the most significant variables affecting the biosurfactant production by *Streptomyces* isolate 5S. In further studies, these five variables will be subjected to Response Surface Methodology (RSM) to confirm the optimized production medium for the high yield of the biosurfactant production by *Streptomyces* sp.

## Conclusion

Although biosurfactants have promising use in bioremediation processes, their industrial scale production is currently difficult due to high raw material costs, high processing costs and low manufacturing output. To optimize the biosurfactant production process, changes need to be made to factors influencing the type and amount of biosurfactant produced by a microorganism. Out of these variables, carbon and nitrogen sources and other physical and chemical parameters such as oxygen, temperature and pH showed to be significant.

Results of the current study showed that, *Streptomyces* sp. 5S, obtained from Egyptian arid soil, favoured molasses, as carbon source, and peptone, as nitrogen source, for the production of biosurfactant. Result of Plackett–Burman design indicated that presence of high levels of peptone, treated molasses, Tween 80, pH, incubation periods and inoculum size in the basal medium positively affected biosurfactant production by *Streptomyces* sp. 5S. Results of ANOVA test and calculated *t*-test showed that treated molasses, incubation periods, Tween 80, inoculum size and peptone were the most significant variables affecting the biosurfactant production by *Streptomyces* sp. 5S.

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